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Cervical Shedding of Cytomegalovirus in Human Immunodeficiency Virus Type 1-Infected Women

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Cervical shedding of cytomegalovirus (CMV) is important in transmission of CMV to exposed sexual partners and neonates. We evaluated prevalence and correlates of CMV DNA shedding in cervical secretions from a large cohort of HIV-1–seropositive women. Using polymerase chain reaction (PCR) assays, CMV DNA was detected in 183 (59%) cervical swab samples from 311 women. Cervical shedding of CMV DNA was significantly associated with shedding of HIV-1 DNA (odds ratio 1.8; 95% confidence interval 1.1–2.8). CMV shedding was also more frequent in women with *Neisseria gonorrhoeae* and *Trichomonas vaginalis* infections, but these associations were not statistically significant. Cervical shedding of CMV in HIV-1–infected women is very frequent and may reflect higher risk of transmission to sexual partners and neonates than previously appreciated. ***J. Med. Virol. 59: 469–473, 1999.***

KEY WORDS: CMV; HIV; genital tract; PCR; female; cervix

INTRODUCTION

Cytomegalovirus (CMV), a member of the herpesvirus family, is transmitted efficiently in immunocompetent populations but generally results in clinically inapparent infection [Ho, 1990]. Seroprevalence studies show CMV infection in 40%–100% of individuals in populations worldwide, with the highest rates generally found in the developing world and in groups of lower socioeconomic status within the developed world [Ho, 1990]. Like other herpesviruses, primary infection with CMV is followed by persistent lifelong infection. During periods of diminished immunity, CMV can reactivate to cause fulminant, occasionally life-threatening, disease, including pneumonitis, colitis, retinitis, and encephalitis [Britt and Alford, 1996]. CMV has become increasingly important as a pathogen in recent decades as the acquired immunodeficiency syndrome (AIDS) and other immunodeficient states, such as posttransplant immunosuppression, have become more prevalent.

Semen and cervicovaginal secretions of CMV-infected individuals are important reservoirs of infectious virus. CMV has been cultured from 27% to 45% of semen samples [Rinaldo et al., 1992; Leach et al., 1993, 1994; Krieger et al., 1995] and detected by polymerase chain reaction (PCR) amplification of CMV DNA in 31%–34% of semen specimens [Rasmussen et al., 1995; Yang et al., 1995]. Similarly, the virus has been cultured from 4% to 20% of cervical swab samples [Chandler et al., 1985; Collier et al., 1995; Clarke et al., 1996] and detected by PCR in 14%–35% of cervical samples [Shen et al., 1993, 1994; Yang et al., 1995; Gradilone et al., 1996]. In adult populations, sexual transmission represents the most common mode of acquiring primary CMV infection [Ho, 1990], and reinfection with multiple strains of CMV is frequently seen in highly sexually active populations [Chandler et al., 1987; Leach et al., 1994]. In CMV-infected women, shedding of CMV in the genital tract also results in frequent transmission to neonates exposed to cervical secretions during delivery [Ho, 1990; Murph et al., 1998].

While shedding of CMV in the semen of HIV-1–infected men has been studied extensively [Rinaldo et

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al., 1992; Leach et al., 1993, 1994; Krieger et al., 1995; Rasmussen et al., 1995; Speck et al., 1999], there has been considerably less work addressing genital tract shedding of CMV in women with HIV-1 [Clarke et al., 1996]. We recently conducted a study to evaluate genital tract shedding of HIV-1 among a cohort of women in Mombasa, Kenya [Mostad et al., 1997]. The seroprevalence of CMV in this population was nearly 100%, which afforded the opportunity to evaluate the frequency and correlates of CMV shedding in the cervical secretions of HIV-1–infected women.

MATERIALS AND METHODS

Study Population and Design

This research was approved by human subjects review committees at the University of Washington (U.S.) and the University of Nairobi (Kenya). HIV-1–seropositive women attending a municipal sexually transmitted diseases clinic in Mombasa, Kenya, were recruited for a cross-sectional study of HIV-1 DNA shedding in cervical and vaginal secretions, as previously described [Mostad et al., 1997]. Briefly, each woman gave informed consent to participate and was interviewed using a standardized questionnaire. She subsequently underwent venipuncture, physical examination, screening for STDs, and collection of cervical fluid samples for viral DNA assays. All participants were enrolled and examined by a single investigator (S.B.M.). At study entrance, sera were tested for antibodies to HIV-1 using two EIAs (Detect; BioChem Immunosystems, Montreal, Canada; and Recombigen; Cambridge Biotech, Worcester, MA). After completion of the original study, stored serum samples were tested for antibodies to CMV (Gull Laboratories, Salt Lake City, UT). The following analysis includes women who were seropositive for both HIV-1 and CMV.

Collection of cervical secretions for viral DNA analysis was accomplished by inserting a dacron swab 1 cm into the cervical os and gently rotating it two full turns. Swabs were placed in dry cryovials and stored for up to 4 hr on ice before transfer to −70°C. Gram stain assessment of cervical polymorphonuclear (PMN) leukocyte count, *Neisseria gonorrhoeae* cultures, *Chlamydia trachomatis* antigen tests, and assessment of *Trichomonas vaginalis* infection were all conducted as previously described [Mostad et al., 1997]. CD4 lymphocytes were quantified in EDTA-anticoagulated blood (Cytosphere; Coulter, Hialeah, FL). Serum estradiol and progesterone levels were determined using EIAs (Immunlite; Diagnostic Products, Los Angeles, CA). Serum vitamin A concentrations were measured with high-pressure liquid chromotography.

CMV DNA Detection

Cervical swab samples were shipped to the University of Washington and were initially assayed for HIV-1 DNA as described [Mostad et al., 1997]. Sample lysates remaining from these initial assays were stored at −70°C and assayed for CMV DNA using PCR assays described elsewhere [Wald et al., 1997; Ryncarz et al., 1999]. PCR primers and probe for CMV detection were directed at the immediate early gene exon 1 [upstream primer (CMV 431) 5′-CCGCGTTCCAATGCACCGTT-CC-3′; downstream primer (CMV 559) 5′-AGGCGTG-TACGGTGGGAGGTCT-3′; probe 5′-CCATAGAAGA-CACCGGGACCGATCCAGCCT-3′]. This region of the immediate early exon gene is highly conserved. The assay was sensitive to the detection of < 5 copies of CMV DNA per 20 μL of specimen.

Data Analysis

Univariate analyses were conducted using Mann-Whitney U, chi-square, and Fisher's exact tests. Logistic regression was used to obtain adjusted estimates for the key univariate predictors of CMV shedding and to determine whether these estimates were confounded by other factors evaluated in this study.

RESULTS

Study Population

Among the 314 HIV-1–seropositive participants who were tested, 313 (99.7%) had antibodies to CMV. Cervical swabs were available for assessment of genital CMV shedding from 311 of these women. The distribution of demographic, clinical, and laboratory variables in the study population are shown in Table I. The median age of the study participants was 28 years (range, 18–46), the median number of sex partners in the previous week was 1 (range, 0–6), and the median frequency of sexual intercourse in the previous week was 1 (range, 0–9).

Cervical Shedding of CMV

CMV DNA was detected by PCR in 183 (59%) of 311 cervical swab samples. Univariate correlates of cervical CMV detection are presented in Table I. Neither age nor indexes of sexual activity were significantly associated with CMV DNA shedding from the cervix. The use of hormonal contraceptives and serum concentrations of vitamin A, estrogen, and progesterone were not significantly predictive of CMV detection in cervical secretions.

There was no significant association between clinical signs and symptoms of HIV-1–related disease or depletion of CD4 lymphocytes and cervical shedding of CMV. However, CMV DNA was detected significantly more frequently in women who were shedding HIV-1 DNA in cervical samples than in those who were not (65% vs. 52%, $P = 0.02$).

The presence of *N. gonorrhoeae* in cervical fluid and *T. vaginalis* in vaginal fluid were associated with increased odds of detecting CMV in cervical secretions, but these associations were not statistically significant. Neither cervical infection with *C. trachomatis* nor elevated numbers of PMNs in cervical fluid were significantly associated with shedding of CMV in the genital tract.

In multivariate analyses, a base model was formed that included the presence of HIV-1 DNA in the cervi-

TABLE I. Univariate Correlates of CMV in Cervical Secretions of HIV-1–Infected Women[a]

Correlate	% of participants	CMV DNA PCR Positive (n = 183)	CMV DNA PCR Negative (n = 128)	OR (95% CI)	P
Questionnaire data					
Age, median (range)	—	28 (18–42)	29 (19–46)		0.2
Number of sex partners in previous week, median (range)	—	1 (0–3)	1 (0–6)		0.7
Frequency of sex in previous week, median (range)	—	1 (0–7)	1 (0–9)		0.7
Contraception[b]					
None/nonhormonal	65%	117/180	83/126	1.0	
OCP	11%	24/180	11/126	1.6 (0.7, 3.6)	0.3
DMPA	18%	32/180	23/126	1.0 (0.5, 1.9)	1.0
Currently pregnant	5%	7/180	9/126	0.6 (0.2, 1.7)	0.3
HIV signs or symptoms[c]	58%	111/183	69/128	1.3 (0.8, 2.1)	0.2
Blood and serum evaluation					
CD4 lymphocytes/μL					
≥500	37%	61/183	55/127	1.0	
400–499	17%	33/183	19/127	1.6 (0.8, 3.2)	0.2
300–399	16%	30/183	19/127	1.4 (0.7, 3.0)	0.3
200–299	14%	27/183	15/127	1.6 (0.7, 3.6)	0.2
100–199	11%	22/183	11/127	1.8 (0.8, 4.4)	0.2
< 100	6%	10/183	8/127	1.1 (0.4, 3.4)	0.8
Vitamin A, μg/dL[d]					
≥40	35%	60/183	49/128	1.0	
30–39	30%	59/183	35/128	1.4 (0.8, 2.5)	0.3
20–29	21%	36/183	29/128	1.0 (0.5, 2.0)	1.0
<20	14%	28/183	15/128	1.5 (0.7, 3.4)	0.3
Serum estradiol, >100 pg/mL[d]	38%	46/116	29/83	1.2 (0.7, 2.3)	0.5
Serum progesterone, >1.0 ng/mL[d]	51%	62/116	40/83	1.2 (0.7, 2.3)	0.5
Genital tract conditions					
HIV-1 DNA present in cervical secretions	51%	104/183	55/128	1.8 (1.1, 2.8)	0.02
Neisseria gonorrhoeae	7%	17/183	6/128	2.1 (0.7, 6.6)	0.1
Chlamydia trachomatis antigen	5%	8/183	6/128	0.9 (0.3, 3.3)	0.9
Trichomonas vaginalis	10%	22/183	8/128	2.1 (0.8, 5.2)	0.09
Cervical PMNs/1,000 X field[e]					
0–30	72%	130/183	93/125	1.0	
31–50	17%	32/183	21/125	1.1 (0.6, 2.1)	0.8
≥51	10%	21/183	11/125	1.4 (0.6, 3.2)	0.4

[a]OR, odds ratio; CI, confidence interval; OCP, oral contraceptives; DMPA, depo medroxyprogesterone acetate.
[b]The reference group includes 182 women using no contraception, 13 with tubal ligation, and 5 women with intrauterine devices. Five women were using other forms of hormonal contraception and were excluded from analysis of contraceptive practices.
[c]Includes a history of cough >1 month, diarrhea > 1 month, fever > 1 month, weight loss > 5 kg, pruritic rash, or the presence on examination of hairy leukoplakia, thrush, zoster, or maculopapular rash.
[d]Includes only women who were not pregnant and not using a hormonal form of contraception.
[e]Average number of polymorphonuclear leukocytes per 1,000 X field of Gram-stained cervical mucus.

cal swab as a predictor of CMV shedding. The other variables appearing in Table I were added to the model one at a time. None of the added variables had an associated P value < 0.10 and none of the additions resulted in a substantial change in the strength or magnitude of the estimates for HIV-1 DNA. Thus, the final model included only the presence of HIV-1 DNA in cervical secretions as a predictor of CMV shedding (OR 1.8; 95% CI 1.1–2.8).

DISCUSSION

We conducted a large study evaluating concomitant shedding of CMV and HIV-1 in the female genital tract and the first study to use PCR-based detection of CMV in a large cohort of HIV-1–infected women. CMV DNA was detected in 183 (59%) cervical swab samples collected from 311 HIV-1–seropositive women, yielding a CMV shedding frequency approximately two- to threefold higher than rates observed in PCR-based evaluations of CMV shedding in HIV-uninfected cohorts (14%–35%) [Shen et al., 1993, 1994; Yang et al., 1995; Gradilone et al., 1996]. This finding corroborates data from a study based on culture detection of CMV, in which the virus was isolated significantly more frequently from the genital tracts of HIV-1–infected than from HIV-1–uninfected women (20% vs. 4%, $P < 0.001$) [Clarke et al., 1996]. A similar study in men showed that CMV could be cultured twice as frequently from the semen of HIV-1–infected men as from HIV-1–uninfected men (33% vs. 17%, $P = 0.05$) [Rinaldo et al., 1992].

We found cervical shedding of CMV DNA to be significantly associated with simultaneous shedding of HIV DNA. Although this is a novel association in women, concurrent shedding of CMV and HIV-1 in the semen of HIV-1–infected men has been noted previously [Krieger et al., 1995; Speck et al., 1999]. Speck et al. [1999] observed that a positive CMV semen culture was an independent predictor of HIV-1 shedding (OR 3.0; 95% CI 1.2–7.7). Krieger et al. [1995] similarly

observed that a positive CMV semen culture was associated with HIV-1 RNA shedding, although this association was not statistically significant (OR 2.9; 95% CI 0.6–13.3). In vitro data have shown that HIV-1 enhances the replication of CMV and that CMV, in turn, can enhance the transcription of HIV-1 [Skolnik et al., 1988; Ho et al., 1990, 1991]. These data suggest a possible bidirectional interaction of these viruses and potential explanation for the association between genital tract shedding of CMV and HIV-1 observed in both women and men.

In HIV-1–infected individuals, symptomatic CMV disease (e.g., retinitis, colitis, esophagitis) occurs almost exclusively among patients with CD4 lymphocyte counts < 100/μL and is accompanied by increased CMV plasma viral load [Gallant et al., 1992; Bowen et al., 1996; Spector et al., 1998]. In individuals with < 50 CD4 cells/μL, CMV disease is common, occurring in up to 40% over 2 years [Pertel et al., 1996]. As such, we anticipated that decline in CD4 cell count might be associated with shedding of CMV in the cervix. However, CD4 count was not significantly associated with shedding of CMV DNA as detected by PCR. Clarke et al. [1996] found that low CD4 lymphocyte count was highly associated with cervical CMV shedding as detected by culture of the virus. The discrepancy in results between these studies raises the possibility that PCR may be detecting low copy numbers of latent CMV DNA in cervical cells rather than exclusively measuring actively replicating virus.

There is enormous genetic diversity in CMV strains resulting in frequent reinfection in individuals who are highly exposed. Genetic analysis of CMV shedding in semen in one large study showed that 19 (29%) of 65 men had different strains isolated in serial samples [Leach et al., 1994]. Three men initially excreted one strain, subsequently shed a different strain, and had the initial virus identified again at a later visit. A similar study of eight women showed two women who shed different strains of CMV in serial isolates [Chandler et al., 1987]. Due to its properties of latency, reactivation, reinfection, numerous strains, and ability to infect many tissue types, the nature of CMV infection in the genital tract is still not well understood. CMV that is shed in cervical secretions and detected, with either PCR or culture, may reflect a chronic persistent infection, reactivated latent infection, or reinfection from a sexual exposure.

Cervical shedding of CMV was associated with *N. gonorrhoeae* and *T. vaginalis* infections in this study, and with *N. gonorrhoeae* and *C. trachomatis* in other studies [Collier et al., 1995; Clarke et al., 1996]. These STDs may be markers for a recent increase in sexual activity or change of sex partners with concomitant acquisition and replication/shedding of a new CMV strain. Alternatively, these STDs may recruit CMV-infected inflammatory cells to the genital tract or somehow facilitate reactivation of latent CMV infection.

Study Limitations

This study has limitations. Firstly, all women in the study were infected with HIV-1. As such, we do not have a prevalence estimate for cervical CMV shedding in a group of HIV-1–uninfected Kenyan women available for direct comparison. The CMV shedding rate in our cohort was very high compared to HIV-1–uninfected Asian and European cohorts, which may reflect geographical differences in addition to HIV-1 serostatus. Secondly, our study utilized PCR-based detection of CMV DNA, which does not distinguish between active viral replication and latent infection. Thirdly, available sequence information indicates that the immediate early exon gene region targeted by our PCR assay is highly conserved between strains, but we are not aware of sequence data for this region in African strains of CMV. The high CMV DNA detection rate in our study suggests that this region is also highly conserved in African strains; however, we recognize that this evidence is indirect and that our assay may lack sensitivity.

SUMMARY

In summary, we found that HIV-1–seropositive Kenyan women shed CMV in cervical secretions with very high frequency: two to three times that of HIV-1–uninfected women in other cohorts. We also found that shedding of HIV-1 DNA in cervical secretions was significantly associated with shedding of CMV DNA, perhaps operating through reciprocal enhancement of transcription. To date, shedding of CMV in the female genital tract has been largely evaluated in cross-sectional studies. Carefully conducted longitudinal studies will help to better understand the nature of CMV infection in the cervix and to determine whether CMV shedding from the cervix represents persistent infection, reactivation of latent virus, or reinfection with a new strain.

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